W0.5
B73

Borden's

REVIEW OF NUTRITION RESEARCH

UNIVERSITY OF MICHIGAN
MAR 3 1958
MEDICAL LIBRARY

Vitamin B_6 in Nutrition and Metabolism

VOLUME 18 · NO. 5
SEPT. - OCT. 1957

BORDEN'S REVIEW

of

NUTRITION RESEARCH

Reviews and summaries of research information concerning nutrition and foods. Published bi-monthly, by THE BORDEN COMPANY; officers of the Company: Harold W. Comfort, president; E. L. Noetzel, treasurer; D. T. Orton, secretary. Editorial office: 350 Madison Avenue, New York 17, N. Y.

Vitamin B_6 in Nutrition and Metabolism

By WILLARD A. KREHL, M.D., Ph.D.*

Introduction

Progress in our knowledge of vitamins seems to wax and wane in a cyclic, tide-like fashion. This has been true for pyridoxine or the vitamin B_6 group of vitamins. The present resurgence of interest in vitamin B_6 is stimulated by recent studies showing that this group of vitamins has an important place in human nutrition and serves as a key link in the metabolism of amino acids and fatty acids. It is appropriate, therefore, to review these developments.

Historical Background

About 1934, Gyorgy proved the existence of a distinct vitamin essential for rats, which was called vitamin B_6. A crystalline compound active in curing vitamin B_6 deficiency symptoms was isolated almost simultaneously in five different laboratories in 1938. Because it was a pyridoxine derivative, the name "pyridoxine" was adopted for it. During 1939, pyridoxine was synthesized in two laboratories. Then pyridoxine and vitamin B_6 became synonymous terms. Later, however, it was shown that the metabolic activity associated with vitamin B_6 could be produced by three naturally occurring compounds: pyridoxal, pyridoxamine and pyridoxine. Since pyridoxine was the name given to a specific compound, technically it should not be used as a synonym for vitamin B_6. That is why this group of three compounds should be referred to as vitamin B_6 or the pyridoxine group.

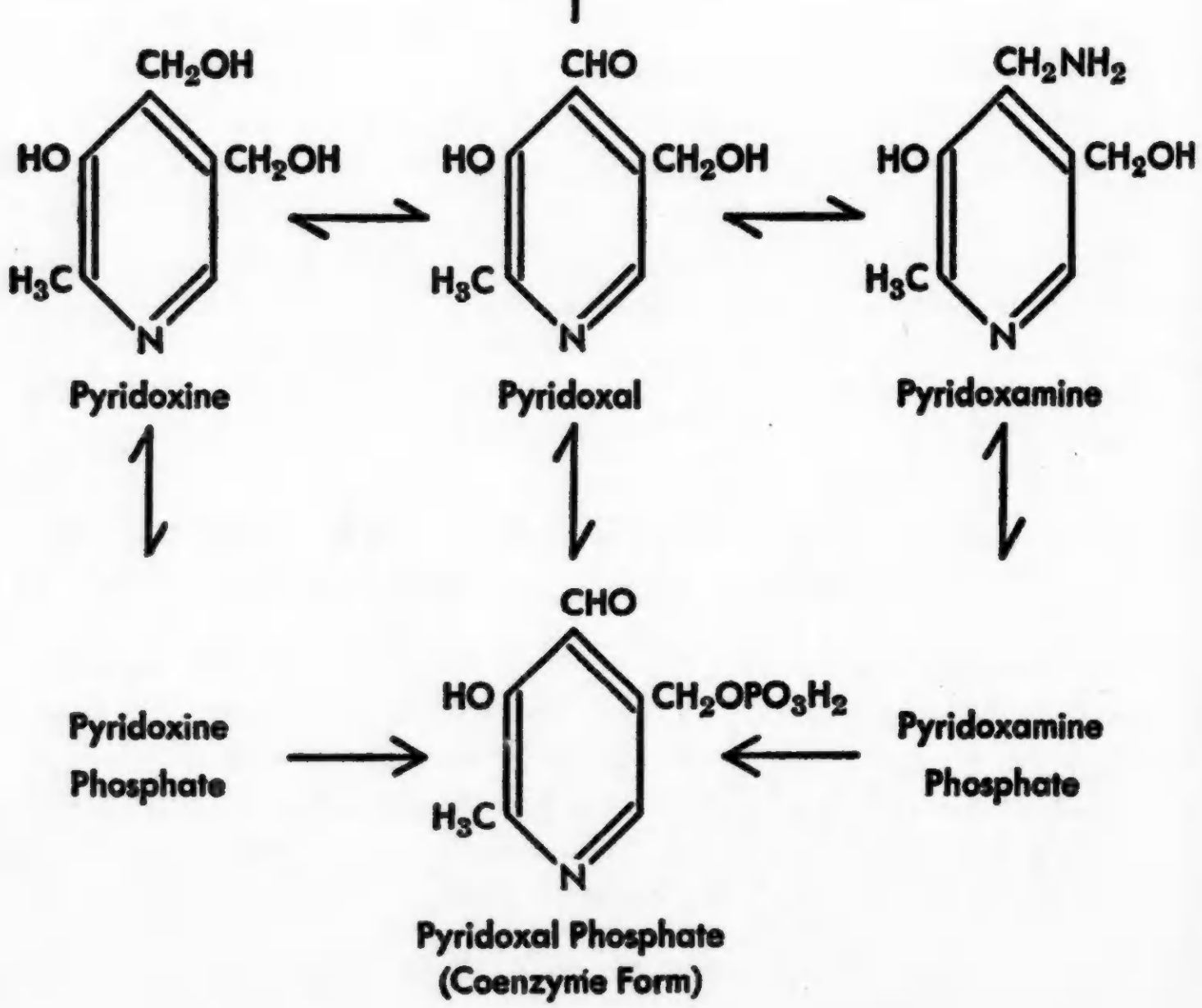
Snell and his associates (2), working with lactic acid bacteria, first recognized the existence of the other natural forms of vitamin B_6. Rats and other animals are capable of converting pyridoxine to these substances which far surpass it in growth-promoting potency for certain lactic acid bacteria. Snell noted further (3, 4), that when pyridoxine was autoclaved with a medium containing amino acids, the growth promoting potency was greatly increased for the test organism *Streptococcus faecalis R.* This increased activity was attributed to the aminated product of pyridoxine, called pyridoxamine. By mild oxidation of pyridoxamine, the formyl derivative, pyridoxal, was obtained. All three forms of vitamin B_6 may occur naturally in the free form and in conjugated or bound forms in natural foodstuffs (5). The bound forms include pyridoxal phosphate

---

*Associate Professor of Nutrition, Yale University Nutrition Laboratory, New Haven 11, Connecticut

and pyridoxamine phosphate, which are further combined with the proteins of foods and biological materials. Although these three forms of the vitamin have approximately equal metabolic activity for animals, they show markedly different activity for different microorganisms (6). This difference in activity for selected microorganisms provides the basis for the assay of the various forms of vitamin B_6. Generally, in natural food products, pyridoxamine, pyridoxal and their corresponding bound forms are present in concentrations greater than pyridoxine

Figure #1 Relationship of the members of the Vitamin B_6 Group (8).



itself. Pyridoxine, however, is more resistant to heat than the other natural forms of vitamin B_6 (7), which, as will be seen later, plays an important part in the preservation of vitamin B_6 activity of foods used for human consumption.

Pyridoxal Phosphate — The Active Form of Vitamin B_6

All of the evidence to date indicates that vitamin B_6 exerts its biological activity by being converted into phosphorylated compounds, principally pyridoxal-5-phosphate (5). This is the prosthetic group or coenzyme of a wide variety of specific catalytic proteins. These phosphate esters or bound forms of vitamin B_6, may be hydrolyzed either by dilute acid or the enzyme phosphatase. The structural formulas for the known forms of vitamin B_6 are presented in figure 1.

When pyridoxine, pyridoxal or pyridoxamine are administered to an animal, the end product of their metabolism, pyridoxic acid, is excreted in the urine (see figure 1). It presumably arises from the oxidation of the free forms of vitamin B_6. This process of breakdown has been demonstrated both *in vitro* and *in vivo* studies. Pyridoxic acid itself has no vitamin B_6 activity for either animals or microorganisms (5).

Reactions of Pyridoxal-5-Phosphate

Pyridoxal-5-phosphate is the coenzyme for a number of biochemical reactions of broad metabolic significance, i.e., transamination and amino acid de-carboxylation. More specifically, it is associated with enzymes concerned with the breakdown of the amino acid tryptophane and its synthesis; the breakdown of serine, threonine, and cysteine; the cleavage of thio-esters and several related reactions (5). In all of these cases, pyridoxal-5-phosphate is the active coenzyme. There are several exceptions where pyridoxamine phosphate is the active co-factor in transaminase activity (9). A summary of the types of reactions that are catalyzed by vitamin B_6 enzymes is presented in table 1. It is apparent that nearly all reactions which involve the non-oxidative breakdown of amino acids require vitamin B_6 coenzymes.

The function of pyridoxal phosphate as a coenzyme in reactions involving a great variety of amino acids helps to clarify the earlier observation (11) that high protein diets fed to rats result in an increased requirement for vitamin B_6.

Another interesting role played by vitamin B_6 is its function in the metabolism of tryptophane. It is required for the cleavage of kynurenine, which is formed from tryptophane to produce anthranilic acid and alanine (12). Similar to this reaction is the cleavage of 3-hydroxykynurenine to hydroxyanthranilic acid. This may be the site of action where pyridoxal phosphate is involved in the conversion of tryptophane to nicotinic acid. In vitamin B_6 deficiency, 3-hydroxykynurnine accumulates and is converted to xanthurenic acid. This substance, long known to be an abnormal end-product of tryptophane metabolism, is

excreted in the urine in increased amounts by vitamin B_6 deficient animals and human subjects.

Interesting relationships between vitamin B_6 and fatty acid metabolism have been shown. It was early noted that there was a marked similarity between the dermatitis resulting from deficiencies of essential fatty acids and from deficiencies of pyridoxine. Birch (13) and Salmon (14) in 1938 reported that an acrodynia-like dermatitis was cured by two factors: vitamin B_6 and the unsaturated fatty acids contained in vegetable oil. Later, Engel (15) found that both pyridoxine and a source of essential fatty acids, such as corn oil, were necessary for the optimum action of choline as a lipotropic agent in rats. Other studies have shown that the curative power of linoleic acid in essential fatty acid deficiency in animals is enhanced by the addition of vitamin B_6 (16). More recent work

TABLE 1

Vitamin B_6 Enzymes (10).

DECARBOXYLASES

Model reaction: $RCHNH_2COOH \longrightarrow RCH_2NH_2 + CO_2$

Examples:

L-Ornithine $\longrightarrow$ Putrescine

L-Glutamate $\longrightarrow$ α-Aminobutyrate

L-Histidine $\longrightarrow$ Histamine

L-Tryptophane $\longrightarrow$ Tryptamine

TRANSAMINASES

Model reaction: $RCHNH_2COOH + R^1COCOOH \rightleftharpoons RCOCOOH + R^1CHNH_2COOH$

Examples:

22 different Amino Acids $\rightleftharpoons$ α-Ketoglutarate

7 different Amino Acids $\rightleftharpoons$ Pyruvate

DEHYDRASES AND DESULFHYDRASES

Model Reaction:

$$R\ \underset{|}{CH}\!-\!\underset{|}{CH}\!-\!COOH \xrightarrow{-H_2A} \left(RCH\!=\!\underset{|}{C}\!-\!COOH \xrightarrow{+H_2O} RCH_2COCOOH + NH_3\right.$$
$$\quad\ AH \quad NH_2 \qquad\qquad\qquad\quad NH_2$$

Examples

L-Serine $\longrightarrow$ Pyruvate + NH_3

L-Homocysteine $\longrightarrow$ α-Ketobutyrate + NH_3

L-Cysteine $\longrightarrow$ Pyruvate + H_2S + NH_3

has shown that vitamin B_6 plays an important role in essential fatty acid metabolism by functioning in an enzyme system which converts linoleic acid to arachidonic acid, both essential fatty acids (17, 18). The significance of this will become more evident when the role of vitamin B_6 in atherosclerosis is considered.

Metabolic Effects of Vitamin B_6 in Vivo

Although vitamin B_6 serves as a coenzyme for reactions involving amino acids, there are still unanswered questions regarding its biochemical function in healthy animals. For example, do these alterations in animo acid metabolism explain certain of the anatomical lesions seen, such as the acrodynia found in vitamin B_6 deficient rats? What overall purposes are actually served by these isolated transamination reactions in the metabolic processes of the intact animal? McHenry has reported experiments which attempt to provide some of the answers to these questions (19). He found that the transaminase activity of the liver did not decrease as pyridoxine deficiency developed, but remained fairly constant. He further noted that the total vitamin B_6 content of the liver attains a minimal level comparatively early in the experiment (20). McHenry notes, that while rats deprived of vitamin B_6 show a decreased appetite, a failure to gain weight, and a reduction in fat storage, none of these effects is necessarily specific for a lack of vitamin B_6, since these symptoms also can be seen in animals that are deprived of almost any nutrient. McHenry's studies have failed to demonstrate any gross failure in protein synthesis or maintenance in vitamin B_6 deficient rats. Blood proteins are not decreased, the total protein content in the carcass is not lessened, although the deprived animal is in somewhat less positive nitrogen balance (21). It may be that vitamin B_6 in the transamination and the decarboxylation reactions of amino acids functions at a more subtle biochemical level than can be measured by the usual methods of evaluating protein metabolism. McHenry has postulated, for example (19), that a decrease in transaminase activity would lessen this form of amino acid metabolism and shunt greater amounts of amino acids into other metabolic routes, for example, by deamination. This concept could explain, for instance, the increased production of urea, and possibly the abnormal metabolism of tryptophane to form xanthurenic acid. It is evident that a correlation between the biochemical alterations of vitamin B_6 deficiency and the anatomical lesions seen remains an enigma.

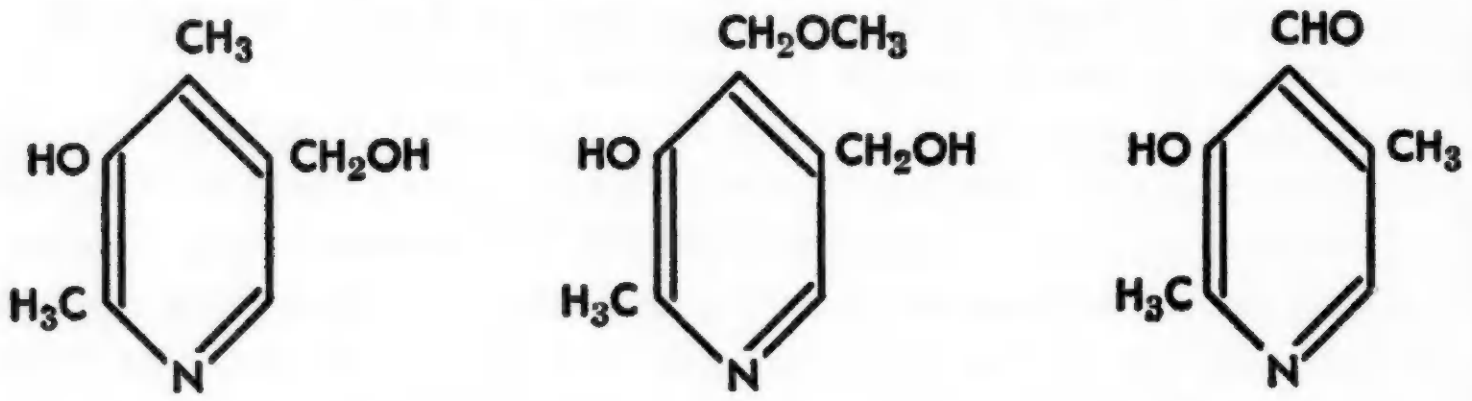
Vitamin B_6 Antagonists

A whole new field in nutrition and biochemistry has grown in the last ten years with the development of metabolic antagonists or antimetabolites. These are substances that bear a structural chemical similarity to the vitamin or nutrient in question, but when antimetabolites are administered, the active vitamin is prevented from exerting its normal function in metabolism. The preparation, therefore, of pyridoxine antimetabolites has provided the experimentalist with

a very useful tool with which to produce and study vitamin B_6 deficiencies (8). This has been of special value in studying vitamin B_6 deficiency in human subjects. A vitamin B_6 deficiency can be produced by feeding a diet deficient in this vitamin, but it is a time-consuming method. The effect can be achieved more rapidly by feeding a vitamin B_6 antagonist.

The structures of the more commonly used vitamin B_6 antagonists are shown in figure 2. Desoxypyridoxine is the compound that has been most extensively used and will be discussed here.

Figure #2 Pyridoxine Antagonists (8).



When desoxypyridoxine is fed in the diet, skin lesions and other characteristic signs of vitamin B_6 deficiency develop. They are usually rapidly reversible by the administration of vitamin B_6. Furthermore, when vitamin B_6 is incorporated in normal amounts in the diet, the antagonistic action of desoxypyridoxine is prevented. One interesting difference, however, is noted when vitamin B_6 deficiency is induced by feeding desoxypyridoxine, there is no reduction in the vitamin B_6 content of the liver and other organs as usually occurs when the deficiencies are induced by a vitamin B_6 deficient diet. With the acute vitamin B_6 deficiency induced by desoxypyridoxine, the amount of pyridoxal phosphate remains high in the tissues, and the activity of the transaminases in liver and heart and the glutamic-decarboxylase in the brain remain essentially unaltered, unless the deficiency is extreme (22, 23, 24). A detailed discussion of desoxypyridoxine-produced deficiency is beyond the scope of this review and is presented elsewhere (8). Desoxypyridoxine is phosphorylated, and while the phosphate itself does not displace pyridoxal phosphate from the enzymes once the latter has been firmly attached, still desoxypyridoxine phosphate can compete with pyridoxal phosphate when both have an essentially equal chance of reaching the enzyme.

Vitamin B_6 Requirements

Analyses of hospital diets have indicated that a daily intake of between 1.0 and 1.5 mg. of pyridoxine is available in the usual daily diet. Estimates of the human daily requirements of vitamin B_6 range between 1 and 2 mg. These

figures are based in part on studies done by Vilter et al in vitamin B_6 deficiency produced in humans by the use of desoxypyridoxine (25).

Urinary excretion of pyridoxic acid, the end product of vitamin B_6 metabolism, varies with the dietary intake of the vitamin, but is usually in the range of 1.5 to 2.0 mg. per day. Approximately 0.8 mg. of vitamin B_6, derived primarily from the microbial flora, is excreted each day in the stool, and probably not available to the human host. Rats and other animals, however, may be able to use a considerable amount of the vitamin B_6 produced by bacterial synthesis in the intestine (26). The amount produced may be considerably reduced by adding antibiotics to the diet (27). It is difficult to evaluate the status of vitamin B_6 nutrition by determining blood levels of this vitamin, since it is present in such low amounts (28). Better data can be obtained by analyzing the pyridoxine content of white blood cells. The status of vitamin B_6 nutrition also can be determined by measuring the level of xanthurenic acid excreted in the urine. As indicated before, during vitamin B_6 deficiency there is an abnormality of tryptophane metabolism and increased amounts of xanthurenic acid are excreted (29, 30). Studies with infants indicate that they should receive 0.2 to 0.3 mg. of vitamin B_6 daily, and this is provided by the usual evaporated milk mixture. Infants with convulsions, where the cause is not determined, should be given 5 to 10 mg. of pyridoxine hydrochloride daily, since some infants have unusually high requirements for this vitamin.

Experimental Vitamin B_6 Deficiency in Human Subjects

Frank vitamin B_6 deficiency states have not been produced in adult humans by feeding pyridoxine deficient diets alone, even after a deficient regimen of 64 days (31). Vilter, however, has produced pyridoxine deficiency very successfully by feeding desoxypyridoxine to human subjects either on a pyridoxine deficient diet or on normal hospital diets (25). The deficiency signs in both cases could be completely reversed by pyridoxine, pyridoxal or pyridoxamine, and partially by the essential fatty acid, linoleic acid. Vitamin B_6 deficiency in the patients was characterized by irritability, depression, and the development of seborrheic dermatitis, which occurred in the nasolabial folds, in the eyebrows and around the eyes in 29 of 50 subjects. Lymphopenia occurred regularly, but anemia could not be related to the vitamin B_6 deficiency state. When tryptophane was administered to vitamin B_6 deficient patients, large amounts of xanthurenic acid appeared in the urine. It was noted that infections developed more frequently in the deficient patients and there was generally a poor response to the antibiotics used to treat the infections, although the organisms present were sensitive to the antibiotics. The infections responded well, however, to antibiotics after vitamin B_6 was administered. These studies provide clear-cut evidence that vitamin B_6 deficiency as induced in the human being by desoxypyridoxine is similar in many respects to the syndrome observed in laboratory animals. Linoleic acid, which partially relieves the skin lesions, had no particular effect on the other aspects of the vitamin B_6 deficiency syndrome. This skin response in humans suggests a

relationship between linoleic acid and pyridoxine to essential fatty acid metabolism, which has also been shown in experimental animal studies. Vilter's studies are significant not only for the production of experimental vitamin B_6 deficiency, but also because they indicate that spontaneous vitamin B_6 deficiency states probably occur rarely on ordinary human dietaries. Diets that are very low in essential fatty acids, high in proteins, and low in members of the B complex, might produce such a deficiency. Diets that have had severe heat processing might also be deficient in vitamin B_6. These circumstances would, however, be most unusual.

Vitamin B_6 and Neurological Function

Of great interest is the ability of pyridoxine to relieve the neuritis which often develops when huge doses of isonicotinic acid hydrazide are used in the treatment of tuberculosis (32), without impairing the antitubercular efficiencies of the drug. A substantial number of people receiving 20 mg. per kg. of this therapeutic agent develop marked neuritis, which was relieved by the administration of 50 mg. of pyridoxine daily.

In 1950, Snyderman et al (33) demonstrated that the nervous systems of children given a diet deficient in vitamin B_6 were adversely affected. These studies involved only two children. Thus, little attention was paid to this work until 1954 when a rather widespread occurrence of nervous irritability and convulsive seizures in 6 weeks to 6 months old infants was reported (34, 35, 36, 37). The convulsive seizures occurred in infants fed a proprietary liquid canned milk formula. The seizures were corrected dramatically by the administration of pyridoxine. It soon became clear that the vitamin B_6 content of the formula had been impaired during manufacture. While marked electroencephalographic alterations were noted in infants during vitamin B_6 deficiency-induced seizures, there was a rapid return to normal in minutes after the injection of the vitamin. This interesting turn of events led to a review of all the previous reports on the effect that vitamin B_6 exerts on the central nervous system. In 1944, Kugelmass (38) reviewed the nutritional basis for nervous disorders in children. He related vitamin B_6 deficiency to symptoms of weakness, irritability, nervousness, ataxia, and abdominal pain. With the increasing awareness of the seriousness of mental disease as a major public health problem, studies involving the pyridoxine group offer a new and possibly fertile area of investigation. From the work that has been conducted so far, it would seem that the critical pyridoxine level for infants is between 50 and 80 mgs. per day.

Tower (39) in a review of the neurochemical aspects of pyridoxine metabolism and function, points out that pyridoxine in its coenzyme form is essential to the activity of a number of important enzyme systems in the brain and that a deprivation of pyridoxine results in epileptiform seizures in all species including man. It is possible that a deficiency of vitamin B_6 is associated with disturbances in the metabolism of neurolipids involving the myelin structure of nerves. The actual mechanisms involved in impairment of nerve function remain unknown.

Since neurolipids comprise 40 to 65 per cent of the total solids of nervous tissue, it is evident that any factor that alters their development or metabolism may seriously involve the normal function of nerve cells. This recalls the early animal studies involving vitamin B_6 deficiency, where a relationship between pyridoxine and essential fatty acid metabolism was recognized. Sherman (40) has concluded from his work that in the pyridoxine-deficient rat, there is a sparing of the breakdown of unsaturated fatty acids with the preferential metabolism of the more highly saturated ones.

About 60 per cent of the non-protein nitrogen content of brain tissue is alpha amino compound and approximately 80 per cent of these are glutamic and aspartic acids and related compounds (41). It is possible that the unusually high concentrations of glutamic acid and glutamine in brain tissue as compared with other organs has physiological importance in the metabolism of nervous tissue (42). Brain tissue contains a special system for the decarboxylation of glutamic acid to form gamma-amino butyric acid (43). This unique system for central nervous system tissue is not present elsewhere. Gamma-amino butyric acid is further metabolized by brain via a transamination reaction to succinic acid, an important component of the Krebs citric acid cycle (44). The original conversion of glutamic acid to gamma-amino butyric acid and the subsequent formation of succinate are catalyzed by pyridoxal phosphate as the coenzyme (43). This again emphasizes the potential role that the pyridoxine group may play in the metabolism of brain tissue. In pyridoxine deficiency, seizures are common and brain excitability is increased, suggesting that maintenance of transamination is essential for normal brain activity. In addition, evidence for abnormalities in glutamic acid and glutamine metabolism have been reported in pyridoxine-deficient animals (39). However, from the studies that have been done on the relationship between pyridoxine and epileptiform seizures, it is evident that there is not a simple, clear-cut relationship, except, perhaps, when there is a marked deficiency of vitamin B_6, especially in small children who are much more prone to the development of seizures. Efforts to demonstrate evidence of pyridoxine-group deficiency in patients with seizures by giving test loads of tryptophane and measuring xanthurenic acid excretion have been unrewarding.

Vitamin B_6 Deficiency and Atherosclerosis

The development of atherosclerosis in both experimental animals and human subjects has generally been related to increased levels of serum cholesterol and/-or beta lipoproteins, particularly those of low density. Current studies on this problem indicate that the dietary intake of unsaturated fatty acids may have an influence in controlling the level of circulating blood cholesterol. It has been suggested that cholesterol when esterified with unsaturated fatty acids is less apt to be deposited in the arterial wall in the form of atheromatous plaques. While the equation Cholesterol = Atherosclerosis is a very attractive one, there is con-

siderable evidence that other factors may be involved and that atherosclerosis may well be a "multi-faceted disease" (45).

Some very interesting studies initiated in 1949 by Rinehart and his group (46) indicate that the pyridoxine group may be intimately involved in the pathology of atherosclerosis. These workers placed a small series of Rhesus monkeys on a pyridoxine deficient diet. Not only did these animals develop much of the classical vitamin B_6 deficiency syndrome, but in addition there occurred degenerative vascular lesions, which were essentially similar to the experimental vascular lesions seen in atherosclerosis in man. These important observations have been continued and expanded considerably (47).

Larger groups of monkeys were placed on vitamin B_6 deficient diets, synthetic in character, containing sucrose, casein, mineral salts, adequate levels of vitamins, with the exception of the pyridoxine group and only 2 per cent of corn oil as a source of fat or essential fatty acids. The control monkeys received the same diet, supplemented with an equivalent of 1 mg. of pyridoxine per day. After 2 to 4 weeks on the deficient regimen, the monkeys began to lose weight and continued a progressive decline in weight throughout the balance of the period of deprivation. The survival time in uncomplicated vitamin B_6 deficiency ranged between 6 to 12 months. Other than a mild decrease in food consumption, a loss in weight and some increased irritability, the animals showed little visual outward appearance of deficiency signs until 5 to 6 months had passed. They then developed unkempt coats, were sluggish in movements, and showed marked hyperirritability. Their hair became shaggy, lost its lustre and was thin.

Anemia was seen almost uniformly by the second month and was unremitting until the animal either died or received vitamin B_6. This anemia was characterized by mild hypochromia with an increase in the cell diameter and decrease in mean cell thickness. Target cells also appeared. The total leucocyte count dropped in 50 per cent of the animals, which had been deficient for an average of 13 months. There was also a decrease in the transaminase and vitamin B_6 levels of the blood.

Three principal pathologic changes were noted in the pyridoxine-deficient Rhesus monkeys. These are: degenerative changes in the arteries; an increased incidence of dental caries; and fatty infiltration of the liver with the early development of cirrhosis. These findings were remarkably constant. Because of their similarity to pathological findings in human subjects, they hold great potential importance with the great current interest in atherosclerosis and heart disease, these findings of degenerative arterial lesions, which very closely simulated those of human arteriosclerosis, assume special significance. Here is evidence that a nutritional factor or factors other than fat, fatty acids or cholesterol directly, may be important in the development of atherosclerosis. Confirmation of these observations has been made in at least one other laboratory (48).

The pathological changes in the arteries were rather widely distributed in the pyridoxine deficient monkeys. The sites of predilection are: (1) the abdom-

inal, iliac, and femoral arteries; (2) arteries in the testicular tunic; and (3) small and medium size branches in the renal arteries. In many animals, similar lesions were found in other vessels, such as the coronary and mesenteric arteries and arteries of the pancreas and other viscera. The authors concluded that suboptimal intakes of the pyridoxine group experimentally maintained over long intervals result in the evolution of atherosclerotic lesions of the type comparable to those seen in man.

In addition to the arteriosclerotic changes noted, severe fatty infiltration of the liver developed in animals in which the deficiency state had been maintained for periods of 3 to 8 months. More extended periods of the deficiency resulted in marked scarring of the liver and early development of cirrhosis.

Another fascinating aspect of this study was the development of dental caries in vitamin B_6 deficient monkeys. In short-term experiments, little or no effect of pyridoxine deficiency was noted in the teeth of the animals. In those monkeys which were maintained on the vitamin B_6 deficient diets for periods of two years or longer, there was a frequent appearance of dental caries. Only those animals that had been on the experimental diet for two years or longer were considered. The caries incidence in the vitamin B_6 deficient group amounted to 16 per cent, while in the control group receiving pyridoxine only 9 per cent.

Vitamin B_6 Metabolism in Pregnancy

Wachstein (49) has reported a disturbance of the metabolism of vitamin B_6 during pregnancy. This is related to an abnormally large excretion of xanthurenic acid in pregnant women who are given a test load of tryptophane. This appeared to be the only significant abnormality, since actual levels of vitamin B_6 in skin, blood, and urine were not found different in pregnant and non-pregnant women (50). It may be that the biochemical abnormality of tryptophane metabolism is but a forerunner of more serious clinical manifestations of vitamin B_6 deficiency. When vitamin B_6 was administered in relatively small amounts to pregnant women, normal metabolism of tryptophane was restored.

Vitamin B_6 in Foodstuffs

Generally speaking, vitamin B_6 is widely distributed in foods, the largest amounts being present in yeast and unrefined grains. Since processed cereals are usually low in vitamin B_6, considerable loss must occur during processing. The factors in food processing that cause destruction have not all been determined, but heat seems to be important.

Since vitamin B_6 can exist in several forms, complex problems of analysis arise in determining the B_6 activity of foods, and a completely satisfactory method has not yet been developed.

Because of the importance of milk in infant nutrition especially, the vitamin B_6 activity of milk products is of interest. In a recent study by two methods of

bacterial assay (51), similar but not identical results were shown for fresh, pasteurized and nonfat dry milk. The average B_6 content of fresh milk by the two methods was 0.37 and 0.35 mg. per liter respectively. No significant losses were noted due to drying and storage of dry milk, or to pasteurization.

The vitamin B_6 content of evaporated milk is much more difficult to evaluate. The B_6 activity of fresh milk is due primarily to pyridoxal (52). Heat treatment, such as sterilization, in the presence of amino acids and proteins converts part of the pyridoxal to pyridoxamine. The change may also continue during storage of the product. The pyridoxamine may itself be further converted to another as yet unidentified form of vitamin B_6. Thus by one method of bacterial assay there was 100 per cent retention of vitamin B_6, while the other bacterial assay method indicated a considerable loss of the vitamin. The one method apparently measures the unknown form of vitamin B_6, while the other procedure does not.

Since evaporated milk has long been used in infant nutrition without giving evidence of vitamin B_6 deficiency, it would seem that the human can either utilize this unidentified form of vitamin B_6 or that evaporated milk contains enough of the known vitamin B_6 to meet the infants' requirements despite some conversion of the vitamin during processing.

SUMMARY

The vitamin B_6 group of vitamins includes pyridoxine, pyridoxal, pyridoxamine and the respective phosphate esters. The principal vitamin activity resides in the enzymatically active form, i.e., pyridoxal-5-phosphate. Vitamin B_6 has been found to be required by many animal species, including man. In the 15 years since its discovery, a wide and significant literature on the effects of vitamin B_6 deficiency has been written. These effects included increased brain excitability and seizures, disorders of the skin, alterations in fatty acid metabolism, including the synthesis of essential fatty acids, amino acid metabolism, anemias, impaired antibody formation and more recently, atherosclerosis and dental caries.

Vitamin B_6 has been shown to participate in a wide variety of enzyme systems, particularly those related to the transformation and utilization of amino acids. The most important reactions involved are those of decarboxylation and transamination.

Vitamin B_6 has been shown an essential vitamin in human nutrition and is required at a level of about 1.5 to 2.0 mg. per day. It is distributed in foods, primarily in the bound forms — pyridoxal and pyridoxamine, which may be destroyed by excessive heat processing. Evidence of vitamin B_6 deficiency in the human may first be demonstrated by an alteration in tryptophane metabolism made manifest by an increased urinary excretion of xanthurenic acid. The possible role that pyridoxine may play in brain metabolism opens a whole new field of research in the study of mental diseases, particularly those associated with hyperexcitability.

BIBLIOGRAPHY

1. Gyorgy, P. Nature *133,* 498 (1934).
2. Snell, E. E., Guirard, B. M., and Williams, R. J. J. Biol. Chem. *143,* 519 (1942).
3. Snell, E. E. Proc. Soc. Exp. Biol. and Med. *51,* 356 (1942).
4. Snell, E. E. J. Biol. Chem. *154,* 313 (1944).
5. Snell, E. E. In Sebrell, W. H., Jr., and Harris, R. S., The Vitamins. Academic Press, New York, 1954, Vol. 3, p. 253.
6. Snell, E. E. and Romefield, A. N. J. Biol. Chem. *157,* 475 (1945).
7. Tomarelli, R. M., Spence, E. R. and Bernhart, F. W. Agri. and Food Chem. *3,* 338 (1955).
8. Umbreit, W. W. Symposium on the Antimetabolites. The National Vitamin Foundation Inc. Nutrition Symposium Series No. 11, Aug. (1955).
9. Meister, A., Sober, H. A. and Peterson, E. A. J. Am. Chem. Soc. *74,* 2385 (1952).
10. Snell, E. E. Vitamin B_6 in Human Nutrition. Report of the 10th M and R Pediatric Research Conf., November (1953).
11. Miller, E. C. and Baumann, C. A. J. Biol. Chem. *157,* 551 (1945).
12. Braunshtein, A. E., Goryachenkova, E. V., and Paskkina, T. S. Biokhimiga *14,* 163 (1949).
13. Birch, T. W. J. Biol. Chem. *124,* 775 (1938).
14. Salmon, W. D. J. Biol. Chem. *123,* CIV (1938).
15. Engel, R. W. J. Nutrition *24,* 175 (1942).
16. Gross, P. J. Invest. Dermatol. *3,* 505 (1940).
17. Snell, E. E. Physiol. Rev. *33,* 509 (1953).
18. Tulpule, P. G. and Patwardhan, W. N. Arch. Biochem. and Biophys. *39,* 450 (1952).
19. McHenry, E. W. Nutrition Symposium Series No. 12, August, 1956. The National Vitamin Foundation, New York, New York.
20. Beaton, J. R., Beare, J. L., Beaton, G. H., Caldwell, E. F., Ozawa, G., and McHenry, E. W. J. Biol. Chem. *207,* 385 (1954).
21. Beaton, J. R., Beare, J. L., White, J. M., and McHenry, E. W. J. Biol. Chem. *200,* 715 (1953).
22. Stoerk, H. C. Ann. New York Acad. Sc. *52,* 1302 (1950).
23. Beaton, G. H. and McHenry, E. W. Brit. J. Nutrition *7,* 357 (1953).
24. Caldwell, E. F. and McHenry, E. W. Arch. Biochem. and Biophys. *45,* 466 (1953).
25. Vilter, R. W., Mueller, J. F., Glazer, H. S., Jarrold, T., Abraham, J., Thompson, C., and Hawkins, V. R. J. Lab. & Clin. Med. *42,* 335 (1953).
26. Elvehjem, C. A. J. Am. Dietet. Assn. *22,* 959 (1946).
27. Di Raimondo, F., Angarano, O., Mannino, N., and Trenchese, L. Intern. Zeitsch. f. Vitaminforsch. *24,* 276, 294, 302 (1952).
28. Linkswiler, H. and Reynolds, M. S. J. Nutrition *41,* 523 (1950).
29. Sarett, H. P. J. Biol. Chem. *189,* 769 (1951).
30. Moller, P. Acta. Physiol. Scandinav. *23,* 47 (1951).
31. Hawkins, W. W. and Barsky, J. Science *108,* 284 (1948).
32. Biehl, J. P. and Vilter, R. W. Proc. Soc. Exp. Biol. and Med. *85,* 389 (1954).
33. Snyderman, S. E., Hold, L. E., Jr., Carretero, R., and Jacobs, K. J. Clin. Nutr. *1,* 200 (1953).
34. Coursin, D. B. J. A. M. A. *154,* 405 (1954).
35. Maloney, C. J. and Parmalee, A. H. J. A. M. A. *154,* 405 (1954).
36. Eliot, J. W. Report of the 10th M. & R. Conf., 53-55, Nov. (1953).
37. May, C. D. Pediatrics *14,* 269 (1954).
38. Kugelmass, I. N. Am. J. Digest. Dis. *11,* 368 (1944).
39. Tower, D. B. Nutrition Symposium Series No. 12, Aug. (1956). National Vitamin Foundation, New York, New York.
40. Sherman, H. Vitamins and Hormones *8,* 55 (1950). Academic Press, New York, New York.
41. Weil, Malherbee H. Biochem. Soc. Sympos. *8,* 16 (1952).
42. Waelsch, H. Neurochemistry. C. C. Thomas, Springfield, Ill., 1955, pp. 173-203.
43. Awapara, J., Landua, A. J., Fuerst, R., and Scale, B. J. Biol. Chem. *187,* 35 (1950).
44. Bessman, S. P., Rossen, J., and Layne, E. C. J. Biol. Chem. *201,* 385 (1953).
45. Page, I. H., Stare, F. J., Corcoran, A. C., Pollack, H., and Wilkinson, C. F. Circulation *16,* August (1957).
46. Rinehart, J. F. and Greenberg, L. D. Am. J. Path. *25,* 481 (1949).
47. Rinehart, J. F. and Greenberg, L. D. Nutrition Symposium Series No. 12, pp. 10-17. August (1956). The National Vitamin Foundation, New York, New York.
48. Mushett, C. W. and Emerson, G. Fed. Proc. *15,* 526 (1956).
49. Wachstein, M. and Gudaitis, A. J. Lab. & Clin. Med. *40,* 550 (1952).
50. Vilter, R. W., Biehl, J. P., Mueller, J. F. and Friedman, B. J. Fed. Proc. *13,* 776 (1954).
51. Hodson, A. Z. J. of Agr. and Food Chemistry *4,* 876 (1956).
52. Rabinowitz, J. C. and Snell, E. E. J. Biol. Chem. *176,* 1157 (1948).

Printed in U.S.A.